Supporting Information for:

ZiCo: a peptide designed to switch folded state upon binding zinc
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Contents:

Materials and methods

Peptide synthesis and purification. ZiCo was made on a Pioneer Peptide Synthesis System (Perseptive Biosystems) using standard Fmoc chemistry. Purification was performed by reversed-phase HPLC and peptide identity was confirmed by MALDI-TOF mass spectrometry. Peptide concentrations were estimated in solution by UV absorption at 280 nm (ε =1490 M^{-1} cm^{-1}).

Circular Dichroism. CD measurements were made using a JASCO J-715 spectropolarimeter fitted with a Peltier temperature controller. Peptide solutions were prepared in 50 mM sodium phosphate, pH 7.5, and 50 mM NaCl when stated, and were examined in 1 mm quartz cuvettes. Spectra were recorded at 5 °C using 1 nm intervals, a 1 nm bandwidth and 8 or 16 sec response times. After baseline correction, ellipticities in mdeg were converted to molar ellipticities (degree cm^2 $dmol^{-1}$) by normalizing for the concentration of peptide bonds and pathlength. Thermal unfolding curves were recorded at 222 nm through 1 °C min^{-1} ramps using a 1 nm bandwidth, averaging the signal for 16 s every 1 °C intervals. Several methods were used to estimate the midpoints (T_Ms) of these curves, notably, taking first and second derivatives of the curves, or fitting the curves to sigmoid functions.

Analytical Ultracentrifugation. Sedimentation equilibrium experiments were conducted at 5 °C in a Beckman-Optima XL-I analytical ultracentrifuge fitted with an An-60 Ti rotor. 100 μl peptide solutions at 150–600 μM in 50 mM sodium phosphate buffer, pH 7.5, 50 mM NaCl, without or with equimolar zinc were equilibrated at speeds in the range 25,000 to 44,000 rpm. Data were fit simultaneously assuming either a single, ideal species model, or a monomer-trimer equilibrium model in NONLIN (Johnson, M. L.; Correia, J. J. *Biophys. J.* **1981**, *36*, 575-588). Data simulations were prepared in the Beckman-Optima XL-A/XL-I data analysis software (v6.03). The monomer molecular weight (3231.8 Da) and partial specific volume (0.7456) of ZiCo were calculated from the amino-acid sequence, and the viscosity of the buffer at 5 °C was taken to be 1.008 mg ml^{-1} (Hayes, D. B.; Laue, T.; Philo, J. *Sednterp* **1995–1998**, University of New Hampshire, U.S.A.).

FT-IR. ATR-FT-IR spectra were acquired on a Tensor 27 (Bruker Optics). Spectra were corrected for water vapour using the “atmospheric compensation” function provided by the Opus software (Bruker), followed by subtraction of the buffer spectrum. Gaussian fitting analysis was performed using the "curve fitting" macro with a bandwidth of 6.5 and resolution enhancement factor of 3. As starting points for the fitting were used peak maxima calculated by the second derivative of the spectrum (9 point smoothing, Savitzky- Golay function).

Isothermal titration calorimetry.
Measurements were obtained using a VP-ITC Microcal instrument using two different protocols.

Protocol I: a concentrated $ZnCl_2$ solution in H_2O (5 mM) was titrated into the cell compartment containing 100 μM ZiCo, 50 mM sodium phosphate, pH 7.5, 50 mM NaCl. The same zinc solution in H_2O was titrated into buffer without ZiCo and the heat of dilution was subtracted from the titration data before further analysis.

Protocol II: a solution of 0.5 mM ZiCo in 50 mM sodium phosphate, pH 7.5, 50 mM NaCl was titrated in the same buffer containing 50 μM $ZnCl_2$. To take the heat of coiled-coil dissociation into consideration, a titration was carried out adding 0.5 mM ZiCo into buffer without zinc. The heat of dissociation obtained in this way was first corrected for the heat of buffer dilution (titration of buffer against buffer) and subsequently subtracted to the titration data before analysis. Data were fitted to a one-binding-site model provided by Microcal ITC extension (Origin software).

NMR spectroscopy. Samples for NMR contained 100 μM ZiCo, 50 mM sodium phosphate, pH 7.5, 10% D_2O, either without or with 100 μM $ZnCl_2$. 1D ^1H NMR spectra

were recorded at 5 °C on a Varian Inova600 spectrometer, equipped with a 5mm HCN triple resonance probe and z-axis pulsed field gradients.

RP-HPLC

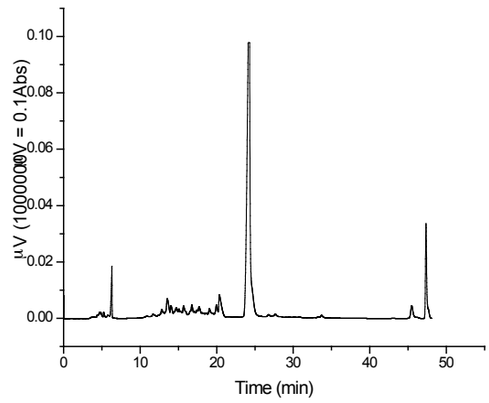

Figure SI1: Semi-preparative RP-HPLC of crude ZiCo peptide.

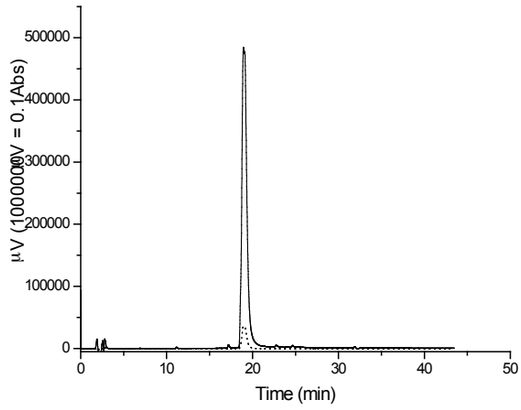

Figure SI2: Analytical RP-HPLC of purified ZiCo.

MALDI-TOF

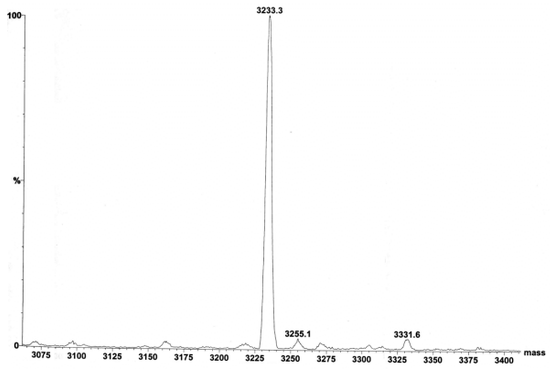

Figure SI3: MALDI-TOF mass spectrometry of the main peak from the RP-HPLC. The expected mass of ZiCo is 3231.8 Da, and the calculated mass of the main peak from RP-HPLC was 3233.3±1.0 Da.

FT-IR

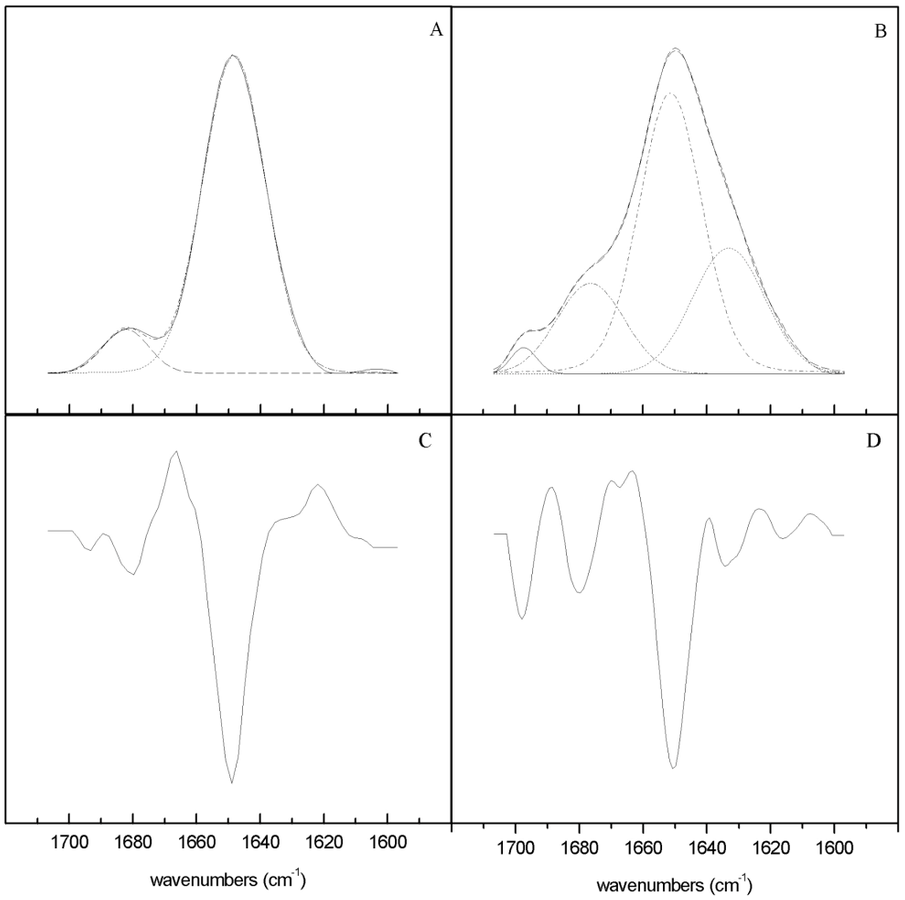

Figure SI4: Infrared (A, B) and second derivative spectra (C, D) of ZiCo in the absence (A, C) and in the presence (B, D) of zinc. In the absence of zinc (A, C), there is a main band at 1649 ± 2 cm^{-1} that can be assigned to α-helical structure and a small component at 1680 ± 2 cm^{-1} that can arise from the fraying of the termini of the helix. In the presence of zinc (B, D), additional bands are present, notably the band at 1633 ± 2 cm^{-1} can be assigned to β-sheet structure.

Analytical Ultracentrifugation

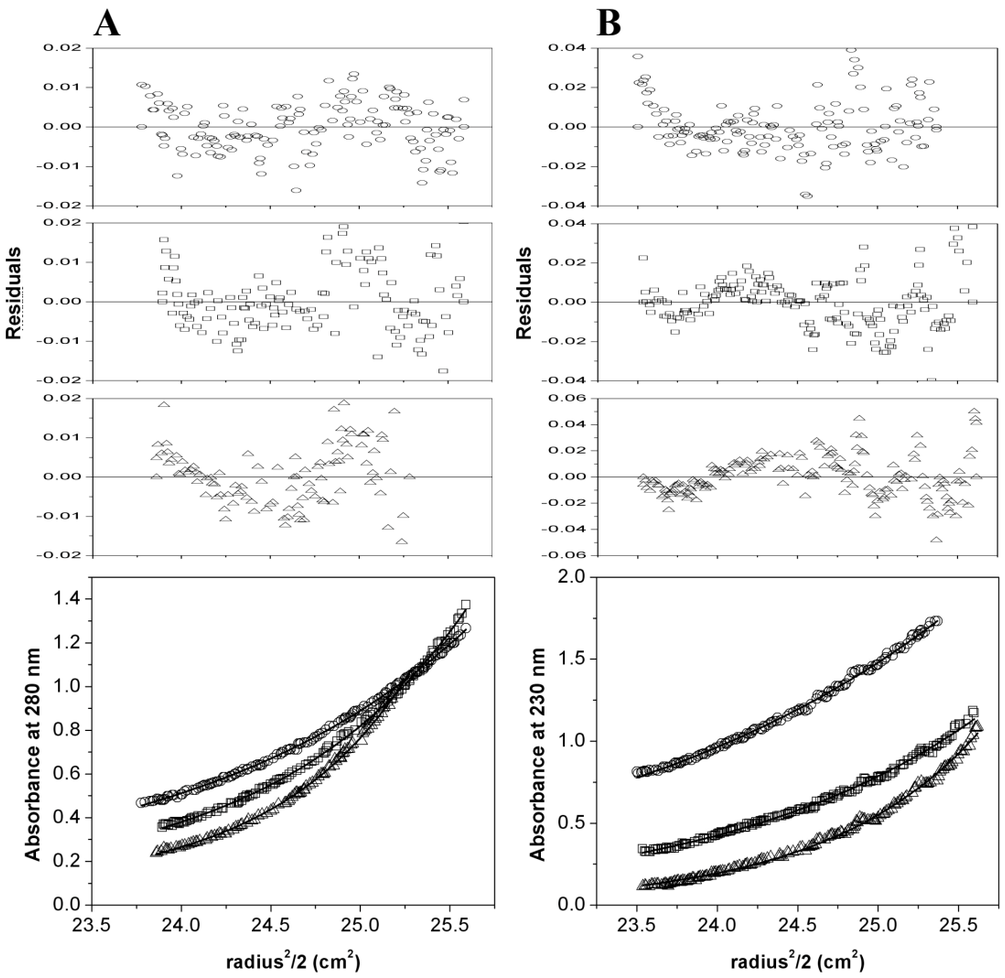

Figure SI5: Analytical ultracentrifugation data of ZiCo (A) without zinc, and (B) with zinc. The bottom panels show sedimentation equilibrium data recorded on ZiCo as plots of absorbance versus radial position ($r^2/2$, cm^2) at three speeds for a given protein concentration at 5 ºC. A: 25,000 rpm (circles), 30,000 rpm (squares), 36,000 rpm (triangles). B: 36,000 rpm (circles), 43,000 rpm (squares), 56,000 rpm (triangles). The fits of these data sets to a model incorporating a monomer-trimer equilibrium (A) or a single species model (B) are also shown. The three upper panels illustrate the residuals for the fits to the three data sets.

Isothermal titration calorimetry

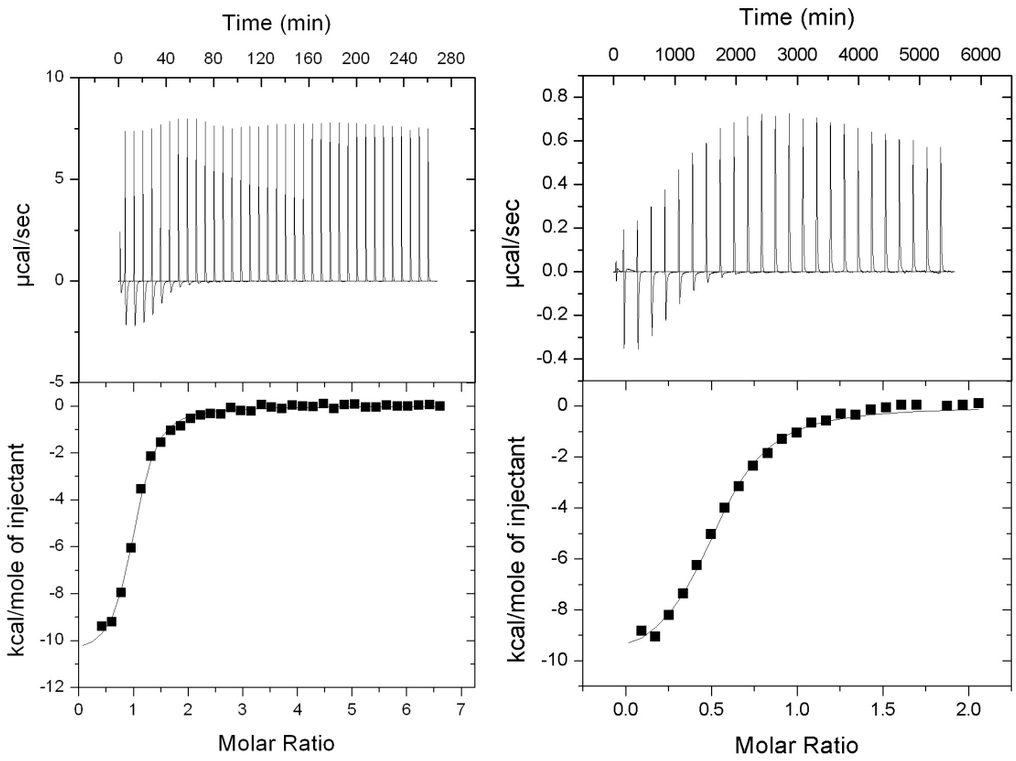

Figure SI6: The left and right panels refer to different protocols for titration. The curve on the left panel was obtained by titrating zinc in a cell containing a solution of the peptide (Protocol I); the titration on the right panel was obtained by subtracting the heat of the coiled-coil dissociation (ZiCo in the syringe and buffer in the cell) from the heat of the titration of a concentrated ZiCo solution into buffer with zinc (Protocol II).

The drawback of the protocol I is the high heat of dilution due to the impossibility of match buffers in the syringe ($ZnCl_2$ has to be prepared in water because $Zn_3(PO4)_2$ is very insoluble) and in the cell. The drawback of the protocol II is, instead, the composite nature of the binding process (coiled-coil dissociation and zinc binding).

	Protocol I	**Protocol II**
N	0.968 ± 0.0105	0.523 ± 0.00956
K_d	4.8 ± 0.4 µM	2.9 ± 0.3 µM
ΔH	-10.75 ± 0.187 kcal mol^{-1}	-10.31 ± 0.253 kcal mol^{-1}
ΔS	-13.7 cal K^{-1} mol^{-1}	-11.1 cal K^{-1} mol^{-1}

NMR spectroscopy

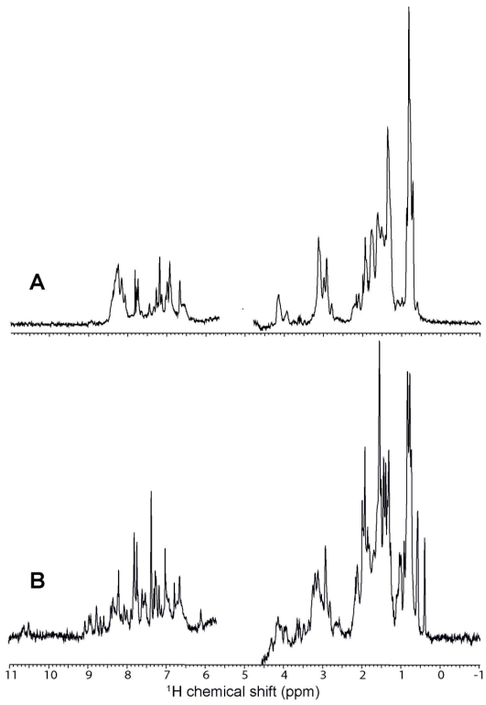

Figure SI7: 1D ^{1}H NMR spectra of 100 μM ZiCo, 50 mM sodium phosphate, pH 7.5, (A) without, and (B) with zinc.